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Rapid Clinical Induction of Hepatic Cytochrome P4502B6 Activity by Ritonavir[▽]

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Ritonavir is the most potent and efficacious inhibitor of cytochrome P4503A (CYP3A), and it is used accordingly for the pharmacoenhancement of other antiretrovirals. Paradoxically, ritonavir induces the clinical metabolism and clearance of many drugs. The mechanism by which ritonavir inhibits and induces clinical drug metabolism is unknown. Ritonavir induces CYP2B6 in human hepatocytes. This investigation tested the hypothesis that ritonavir induces human CYP2B6 *in vivo*. Thirteen healthy human immunodeficiency virus-negative volunteers underwent a three-way sequential crossover protocol, receiving racemic bupropion after nothing (control), 3 days of treatment with ritonavir, and 2.5 weeks of treatment with ritonavir (400 mg twice a day). Stereoselective bupropion hydroxylation was used as an *in vivo* probe for CYP2B6 activity. Plasma and urine (*R*)- and (*S*)-bupropion and (*R,R*)- and (*S,S*)-hydroxybupropion concentrations were measured by liquid chromatography-mass spectrometry. Racemic, (*R*)-, and (*S*)-bupropion plasma ratios of the area under the concentration-time curve from 0 h to infinity ($AUC_{0-\infty}$) (ritonavir/control) were significantly reduced to 0.84, 0.86, and 0.80, respectively, after 3 days of ritonavir treatment and to 0.67, 0.69, and 0.60 after steady-state ritonavir treatment. Apparent oral clearances for racemic, (*R*)-, and (*S*)-bupropion all were significantly increased by 1.2-fold after 3 days of ritonavir treatment and by 1.4-, 1.7-, and 1.5-fold after steady-state ritonavir treatment. The plasma (*S,S*)-hydroxybupropion/(*S*)-bupropion AUC_{0-72} ratio was significantly increased by ritonavir. Formation clearances of both (*R,R*)- and (*S,S*)-hydroxybupropion were increased 1.8-fold after 3 days of ritonavir treatment and 2.1-fold after steady-state ritonavir treatment. These results show that ritonavir induces human CYP2B6 activity. Induction is rapid, occurring after only 3 days of ritonavir, and is sustained for at least 2 weeks. The ritonavir induction of CYP2B6 activity may have significant implications for drug interactions and clarify previously unexplained interactions.

Human immunodeficiency virus (HIV) drugs are notorious perpetrators of drug interactions. The protease inhibitors ritonavir, indinavir, nelfinavir, saquinavir, lopinavir, and amprenavir and the reverse transcriptase inhibitor efavirenz can induce and/or inhibit the metabolism and clearance of numerous drugs (45). Ritonavir is the most potent and efficacious inhibitor of cytochrome P4503A (CYP3A), for which it is both a substrate and a mechanism-based inhibitor (13). Ritonavir is a standard component of highly active retroviral therapy and is included for the pharmacoenhancement of other antiretrovirals (boosting), which is achieved via greater bioavailability and increased and sustained plasma concentrations. Boosting occurs as a result of the inhibition of first-pass and hepatic CYP3A activity and/or the inhibition of P-glycoprotein activity (42).

Although traditionally associated with cytochrome P450 (CYP) inhibition, there is growing evidence that protease inhibitors also can cause CYP induction and attendant drug interactions. Specifically, ritonavir has been shown to induce CYP2B6 mRNA, protein expression, and catalytic activity in human hepatocytes *in vitro* (8, 15). Clinically, ritonavir has been shown to increase the metabolism and/or clearance of drugs such

as meperidine, ethinyl estradiol, olanzapine, trimethoprim-sulfamethoxazole, and methadone (45). Such induction might be attributable to effects on CYP2B6 activity, since this isoform has been shown to metabolize meperidine (44) and methadone (20, 31, 47, 48). Nevertheless, the clinical effects of ritonavir on human CYP2B6 activity are unknown.

The increasing significance of CYP2B6 in human drug metabolism and disposition has been recognized recently, after initially being considered a minor isoform of relative unimportance (12, 26, 49, 54). CYP2B6 catalyzes the bioactivation of the antineoplastic agents ifosfamide, cyclophosphamide, and tamoxifen, the metabolic inactivation of anesthetics and analgesics such as methadone, meperidine, propofol, and ketamine, the biotransformation of abused drugs such as nicotine, ecstasy, and phencyclidine, and pesticides and other environmental contaminants. Notably, CYP2B6 also catalyzes the metabolism of the antiretrovirals efavirenz and nevirapine (7, 46, 49). The proportion of drugs metabolized by CYP2B6 has been estimated to be as high as 8% (39).

If ritonavir is a CYP2B6 inducer, this could explain the increased elimination of several drugs. The purpose of this investigation was to test the hypothesis that ritonavir is an inducer of hepatic CYP2B6 activity in humans. Subjects were studied after both acute and steady-state ritonavir treatment, since there is some evidence that the duration of administration influences enzyme inhibition (short-term ritonavir) and induction (long-term ritonavir) (22); there is clear evidence that the induction of methadone clearance by ritonavir is rapid (30, 34).

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The hydroxylation of (*S*)-bupropion was used as the *in vivo* probe for CYP2B6 activity, since this has been shown to be an improved CYP2B6 probe compared to racemic bupropion (32). Although racemic bupropion clearance or hydroxylation has been used clinically to phenotype CYP2B6 activity (35, 36, 41, 50), racemic bupropion suffers from well-documented limitations. Specifically, bupropion clearance may not reflect altered CYP2B6 activity, because hydroxylation is a minor metabolic pathway and there are alternate non-CYP2B6 routes of bupropion metabolism; also, plasma hydroxybupropion concentrations are elimination rate limited (the metabolite half-life exceeds that of the parent) rather than formation rate limited (36, 50). Both (*R*)- and (*S*)-bupropion were hydroxylated by CYP2B6, but the metabolism was stereoselective (4), and clinically, the apparent oral clearance (CL/F) of (*S*)-bupropion was threefold greater than that of (*R*)- and (*R,S*)-bupropion (32). More importantly, (*S,S*)-hydroxybupropion was formation rate limited, while (*R,R*)-hydroxybupropion and (*R,R*),(*S,S*)-hydroxybupropion were elimination rate limited; hence, (*S,S*)-hydroxybupropion formation is a better *in vivo* CYP2B6 probe (32).

MATERIALS AND METHODS

Study population and protocol. Thirteen volunteers (four males, nine females) participated in the investigation, which was approved by the Washington University Institutional Review Board, after written informed consent was obtained. Inclusion criteria included being (i) 18 to 40 years old and (ii) within 30% of ideal body weight. Exclusion criteria were (i) major medical problems; (ii) a history of liver or renal disease; (iii) the use of prescription medications, drugs, herbs, and foods metabolized by or altering CYP2B6 or CYP3A4; and (iv) a family history of type 2 diabetes. Females taking hormonal contraceptives were excluded from enrollment. Both smokers and nonsmokers were enrolled. Subjects underwent a screening visit, during which blood samples for fasting glucose concentration and HIV serologic status were obtained. Subjects were excluded if their glucose exceeded 110 mg/dl (because ritonavir can cause glucose intolerance) or if they were HIV seropositive (since monotherapy can cause HIV resistance). The final study population was comprised of 13 subjects (29 ± 5 years [range, 23 to 39 years], 67 ± 9 kg [range, 53 to 84 kg]). One additional subject completed the control session but withdrew after starting ritonavir due to nausea, fatigue, and cramping, and the control session data are not included in the data set.

The protocol used a three-session sequential crossover design (with the control session first due to logistical considerations). There was at least a 2-week washout between the first two study sessions. Subjects were instructed to consume no orange-, apple-, or grapefruit-containing foods or juices for 5 days before and on each study day and no alcohol or caffeine for 24 h before and on each study day. An intravenous catheter was placed in an arm vein for blood sampling. Subjects received 150 mg oral immediate-release bupropion on three occasions after no pretreatment (control), on the 3rd day of ritonavir treatment, and on the 17th day of ritonavir treatment. Ritonavir dosing was 200 mg three times daily for 1 day, 300 mg twice daily for the next 6 days, and then 400 mg twice daily. The last ritonavir dose was taken the night before the last blood sample, and the total duration of administration was 18 days. Previous investigations suggested that steady-state induction is achieved after approximately 2 weeks of ritonavir (28, 40, 43). Venous blood samples were obtained for 48 h after bupropion treatment, and plasma was separated and stored at -20°C for later analysis. All urine was collected for 48 h after bupropion treatment as two 24-h collections, the volume was measured, and an aliquot was stored at -20°C for later analysis. Subjects were fed a light breakfast 3 h after bupropion administration and had free access to food and water thereafter.

Analytical methods. Plasma and urine (*R*)- and (*S*)-bupropion and (*R,R*)- and (*S,S*)-hydroxybupropion concentrations were measured by high-performance liquid chromatography-tandem mass spectrometry as described previously (3). Interday coefficients of variation were the following: for 1, 10, and 100 ng/ml (*R*)-bupropion, 9, 9, and 8%, respectively; for 1, 10, and 100 ng/ml (*S*)-bupropion, 10, 9, and 10%, respectively; for 5, 50, and 500 ng/ml (*R,R*)-hydroxybupropion, 10, 5, and 8%, respectively; and for 5, 50, and 500 ng/ml (*S,S*)-hydroxybupropion, 8, 6, and 3%, respectively.

Pharmacokinetic analysis. Data were analyzed using noncompartmental methods (WinNonlin 5.1; Pharsight Corp., Mountain View, CA) as described previously (33). The CL/F was the ratio of the dose to the area under the concentration-time curve (AUC), and the apparent renal or formation clearance was calculated as the product of the fraction of the dose recovered in urine and CL/F .

Sample size. For statistical power calculations, we consider a simplified analysis (paired *t* test) for comparing control and HIV drug treatments that was based on the primary outcome variable: the plasma hydroxybupropion/bupropion AUC ratio. Intraindividual variability in this parameter has not been determined; hence, it could not be used for sample size calculation. Nevertheless, experience with within-subject (interday) variability in the clearance of other P450 probes suggested that the intraindividual coefficients of variation typically were 30 to 40%. For a primary outcome variable, detecting a 30% change (standard deviation [SD], 35%; α , 0.05; β , 0.8) would require 13 subjects. Furthermore, previous induction studies using 12 subjects were adequately powered to detect induction (30).

Statistical analysis. Results are expressed as the means \pm SD or median. Analysis of variance was used to assess the significance of differences between treatments (SigmaStat 3.5; Systat Corp). Nonnormal data were log transformed for statistical analysis. Significance was assigned at $P < 0.05$. Differences between treatments in clearances and AUC ratios also were assessed as the geometric mean ratios (ritonavir/control) and the 90% confidence intervals of the geometric means. Confidence intervals excluding unity were considered statistically significant.

RESULTS

One subject withdrew (for scheduling reasons) during the steady-state ritonavir phase of the protocol; thus, data were not obtained for this subject session. Results are presented for 13 subjects (control and 3 days of ritonavir) and for 12 subjects (2.5 weeks of ritonavir).

Ritonavir caused significant changes in bupropion disposition. Plasma concentrations of both (*R*)- and (*S*)-bupropion were diminished after both 3 days of ritonavir treatment and steady-state ritonavir treatment (Fig. 1). The ratios of plasma AUC from 0 h to infinity ($AUC_{0-\infty}$) (ritonavir/control) for racemic, (*R*)-, and (*S*)-bupropion were significantly reduced to 0.84, 0.86, and 0.80, respectively, after 3 days of ritonavir treatment and to 0.67, 0.69, and 0.60 after more than 2 weeks of ritonavir treatment (Table 1). (*R*)-, (*S*)-, and racemic bupropion CL/F s all were significantly increased by 1.2-fold after 3 days of ritonavir treatment and by 1.4-, 1.7, and 1.5-fold after steady-state ritonavir treatment. Short-term ritonavir decreased the plasma $AUC_{0-\infty}$ of total and (*R,R*)-hydroxybupropion but not (*S,S*)-hydroxybupropion, while steady-state ritonavir treatment decreased the plasma $AUC_{0-\infty}$ of total, (*R,R*)-, and (*S,S*)-hydroxybupropion (Fig. 2). The median apparent elimination half-life of total, (*R,R*)-, and (*S,S*)-hydroxybupropion was reduced from 32, 33, and 18 h, respectively, to 17, 18, and 13 h by 3 days of ritonavir treatment and to 11 h for all three by steady-state ritonavir treatment. The plasma hydroxybupropion/bupropion AUC_{0-48} ratios for racemic and (*R*)-bupropion were minimally changed or were unchanged by both short-term and steady-state ritonavir treatments, while the metabolite/parent ratio for (*S*)-bupropion was increased by both short-term and steady-state ritonavir treatments. Ritonavir caused a 1.8-fold increase in the formation clearance of both (*R,R*)- and (*S,S*)-hydroxybupropion after 3 days and a 2.1-fold increase after 2 weeks (Table 2). Similar results were observed for the 0- to 24-h urine hydroxybupropion/bupropion molar ratio. Ritonavir had no effect on bupropion enantiomer renal clearance.

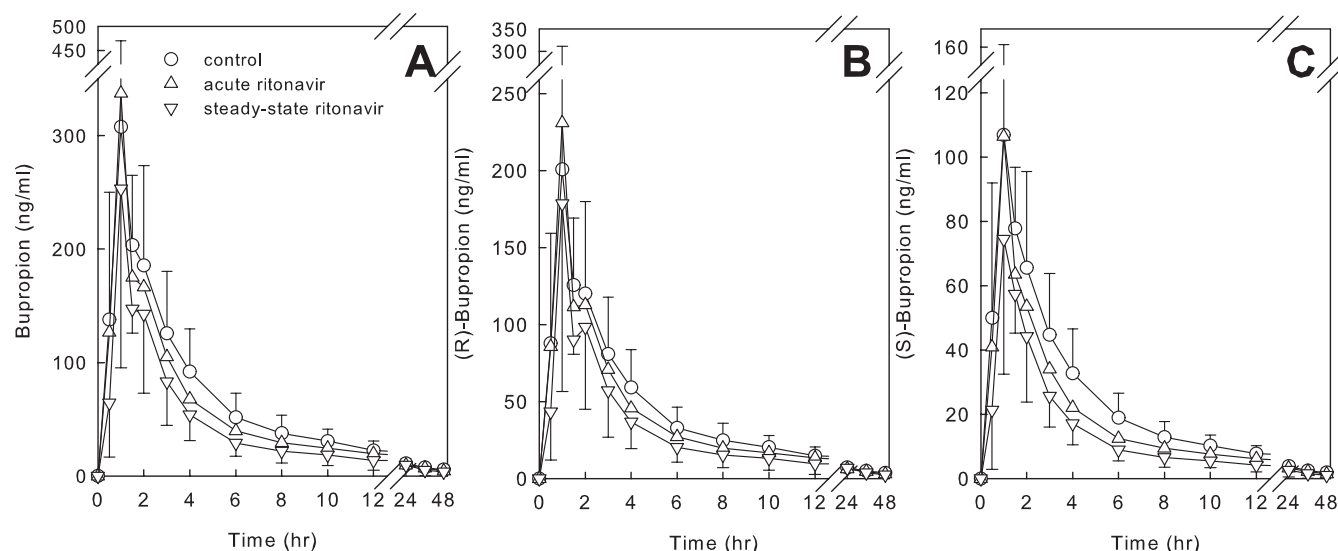


FIG. 1. Plasma concentrations of racemic bupropion (A) and bupropion enantiomers (B and C). Results are the means \pm SD ($n = 13$ for controls and acute ritonavir treatment; $n = 12$ for steady-state ritonavir treatment). Some SD are omitted for clarity.

DISCUSSION

This investigation demonstrates that short-term (3 days) and steady-state ritonavir treatments significantly increased (*S*)-bupropion hydroxylation. Specifically, ritonavir increased (*S,S*)-hydroxybupropion apparent formation clearance, 0- to 24-h urine (*S,S*)-hydroxybupropion/(*S*)-bupropion molar ratios, plasma (*S,S*)-hydroxybupropion/(*S*)-bupropion AUC ratios, and (*S*)-bupropion CL/F . Changes in (*S*)-bupropion clearance were not due to increased bupropion renal clearance. (*S*)-Bupropion hydroxylation is catalyzed selectively by CYP2B6 and an *in vivo* probe for CYP2B6 (32). Therefore, these results strongly support the conclusion that human hepatic CYP2B6 activity *in vivo* is induced by both short-term and steady-state ritonavir treatments, with the latter causing an approximately twofold increase. Although ritonavir also increased apparent formation clearances, urine hydroxybupropion/bupropion molar ratios, and systemic clearance of (*R,R*)- and racemic hydroxybupropion, plasma hydroxybupropion/bupropion AUC ratios were decreased rather than increased. There is insufficient understanding of hydroxybupropion elimination to explain this last observation (32).

One previous investigation evaluated the effects of ritonavir on bupropion disposition (24). Subjects received bupropion (presumably the immediate-release variety, although it was not specified) on the second day of treatment with 200 mg ritonavir twice daily. Racemic bupropion and metabolite plasma concentrations were measured for 24 h. Neither bupropion nor hydroxybupropion concentrations were significantly altered by ritonavir, and the hydroxybupropion/bupropion ratio was insignificantly decreased by 15%, which was interpreted as suggesting no effect on CYP2B6 activity. There are, however, differences between previous studies and the present study. Hesse et al. evaluated racemic bupropion plasma concentrations and the hydroxybupropion/bupropion AUC ratio (24). Nevertheless, plasma bupropion concentrations may be insensitive to altered CYP2B6 activity, because CYP2B6-catalyzed hydroxylation is only a minor route of overall bupropion elim-

ination (36, 50). Additionally, the plasma hydroxybupropion/bupropion AUC ratio may be confounded, because racemic hydroxybupropion is elimination rate rather than formation rate limited, and hydroxybupropion elimination also may be altered (32, 36). Hesse et al. also evaluated bupropion metabolism after fewer and lower doses of ritonavir (three to four doses of 200 mg) than those used herein (3 days of two doses of 200 mg and three to four doses of 300 mg and 2.5 weeks of 400 mg twice daily) and speculated that longer ritonavir exposure might induce CYP2B6. The present investigation demonstrates that both short-term and steady-state ritonavir treatments do induce hepatic CYP2B6 activity.

The ritonavir induction of hepatic CYP2B6 is consistent with previous *in vitro* findings. Ritonavir increased CYP2B6 mRNA expression in LS180 adenocarcinoma cells (23) and induced CYP2B6 mRNA expression, immunoreactive protein expression, and catalytic activity in primary human hepatocytes (8, 15). Ritonavir was classified as a strong *in vitro* CYP2B6 inducer, along with rifampin, phenobarbital, phenytoin, and clotrimazole (15).

Other CYP2B6 inducers also have been shown to induce the metabolism of racemic bupropion and other CYP2B6 substrates. The prototypic CYP2B6 inducer rifampin induced bupropion clearance by threefold and increased peak plasma hydroxybupropion concentrations (36). Lopinavir/ritonavir (400 mg lopinavir/100 mg ritonavir twice daily for 2 weeks) decreased the plasma AUC for bupropion and hydroxybupropion by 57 and 50%, respectively (27). Ritonavir (500 mg twice daily for 10 days) decreased the plasma meperidine AUC and increased the normeperidine AUC, suggesting the induction of metabolism (43). Although this was initially attributed to CYP3A induction (43), subsequent reports showing that meperidine is primarily a CYP2B6 substrate (44) and that ritonavir does not induce CYP3A4 (see below) suggest that the ritonavir enhancement of meperidine metabolism and clearance likely reflects CYP2B6 induction.

The ritonavir induction of hepatic CYP2B6 activity has clin-

TABLE 1. Pharmacokinetic parameters for plasma bupropion and hydroxybupropion^a

Substance and parameter ^b	Results according to treatment type ^c				
	Control (<i>n</i> = 13)	Acute ritonavir (<i>n</i> = 13)		Steady-state ritonavir (<i>n</i> = 12)	
	Mean ± SD	Mean ± SD	Ratio	Mean ± SD	Ratio
Bupropion					
<i>C</i> _{max} (ng/ml)	312 ± 142	345 ± 245		267 ± 143	
AUC _{0-∞} (h · ng · ml ⁻¹)	1,440 ± 520	1,290 ± 700	0.84 (0.72, 0.97)	1,030 ± 660*	0.67 (0.53, 0.83)
CL/ <i>F</i> (liter · min ⁻¹)	1.93 ± 0.61	2.47 ± 1.24	1.19 (1.03, 1.38)	3.24 ± 1.52*	1.50 (1.21, 1.87)
Elimination <i>t</i> _{1/2} (h)	18.7 ± 6.6	16.8 ± 5.8		17.8 ± 6.4	
(R)-Bupropion					
<i>C</i> _{max} (ng/ml)	203 ± 100	235 ± 186		188 ± 114	
AUC _{0-∞} (ng · h · ml ⁻¹)	927 ± 386	866 ± 553	0.86 (0.74, 0.99)	713 ± 517	0.69 (0.56, 0.86)
CL/ <i>F</i> (liter · min ⁻¹)	1.54 ± 0.54	1.96 ± 1.07*	1.17 (1.01, 1.35)	2.51 ± 1.30*	1.44 (1.16, 1.78)
Elimination <i>t</i> _{1/2} (h)	17.6 ± 5.6	15.9 ± 5.5		17.3 ± 5.7	
(S)-Bupropion					
<i>C</i> _{max} (ng/ml)	109 ± 45	110 ± 61		80 ± 35	
AUC _{0-∞} (h · ng · ml ⁻¹)	509 ± 148	423 ± 167*	0.80 (0.69, 0.93)	318 ± 166*	0.60 (0.46, 0.76)
CL/ <i>F</i> (liter · min ⁻¹)	2.65 ± 0.77	3.45 ± 1.46*	1.25 (1.08, 1.45)	4.96 ± 2.32*	1.68 (1.31, 2.15)
Elimination <i>t</i> _{1/2} (h)	19.4 ± 8.1	17.6 ± 6.4		18.4 ± 7.6	
Hydroxybupropion					
<i>C</i> _{max} (ng/ml)	335 ± 148	345 ± 192		330 ± 191	
<i>T</i> _{max} (h)	3.7 ± 1.7	3.6 ± 2.3		3.4 ± 2.3	
AUC ₀₋₇₂ (h · ng · ml ⁻¹)	9,640 ± 4,650	8,470 ± 4,200	0.86 (0.80, 0.93)	6,100 ± 2,870	0.62 (0.51, 0.75)
AUC _{0-∞} (h · ng · ml ⁻¹)	15,490 ± 8,530	11,330 ± 6,050	0.69 (0.59, 0.89)	7,560 ± 4,130	0.43 (0.31, 0.70)
Elimination <i>t</i> _{1/2} (hr)	31.9 ± 8.1	22.6 ± 14.2		18.6 ± 17.4*	
AUC ₀₋₇₂ hydroxybupropion/bupropion (h · ng · ml ⁻¹)	8.4 ± 5.2	9.0 ± 7.4	1.02 (0.90, 1.15)	8.4 ± 6.0	0.92 (0.79, 1.07)
AUC _{0-∞} hydroxybupropion/bupropion (h · ng · ml ⁻¹)	12.0 ± 8.3	10.6 ± 8.4	0.89 (0.76, 1.06)	9.1 ± 5.8	0.73 (0.58, 0.97)
(R,R)-Hydroxybupropion					
<i>C</i> _{max} (ng/ml)	317 ± 139	325 ± 184		311 ± 182	
<i>T</i> _{max} (h)	3.8 ± 1.7	3.8 ± 2.2		3.5 ± 2.2	
AUC ₀₋₇₂ (h · ng · ml ⁻¹)	9,290 ± 4,510	8,120 ± 4,080*	0.86 (0.79, 0.93)	5,829 ± 2,790*	0.61 (0.50, 0.74)
AUC _{0-∞} (h · ng · ml ⁻¹)	15,240 ± 8,450	10,950 ± 5,930*	0.68 (0.58, 0.89)	7,330 ± 4,140*	0.42 (0.30, 0.71)
Elimination <i>t</i> _{1/2} (hr)	33.1 ± 9.1	23.1 ± 15.1*		19.3 ± 19.1*	
AUC ₀₋₇₂ hydroxybupropion/bupropion (h · ng · ml ⁻¹)	12.7 ± 8.0	13.4 ± 11.5	0.99 (0.86, 1.13)	12.2 ± 9.0	0.87 (0.75, 1.01)
AUC _{0-∞} hydroxybupropion/bupropion (h · ng · ml ⁻¹)	18.8 ± 13.5	16.1 ± 13.2	0.86 (0.72, 1.03)	13.4 ± 8.8	0.68 (0.52, 0.94)
(S,S)-Hydroxybupropion					
<i>C</i> _{max} (ng/ml)	20 ± 11	25 ± 14		23 ± 13	
<i>T</i> _{max} (h)	2.2 ± 1.5	1.7 ± 0.8		1.5 ± 0.6	
AUC ₀₋₇₂ (h · ng · ml ⁻¹)	352 ± 206	356 ± 165	1.04 (0.95, 1.14)	274 ± 117	0.81 (0.67, 0.97)
AUC _{0-∞} (h · ng · ml ⁻¹)	420 ± 251	404 ± 180	0.99 (0.90, 1.10)	303 ± 123	0.75 (0.61, 0.92)
Elimination <i>t</i> _{1/2} (h)	18.1 ± 2.8	15.3 ± 6.1		13.9 ± 7.3	
AUC ₀₋₇₂ hydroxybupropion/bupropion (h · ng · ml ⁻¹)	0.84 ± 0.56	1.05 ± 0.69*	1.28 (1.11, 1.47)	1.15 ± 0.67*	1.33 (1.07, 1.66)
AUC _{0-∞} hydroxybupropion/bupropion (h · ng · ml ⁻¹)	0.89 ± 0.60	1.06 ± 0.66*	1.24 (1.11, 1.39)	1.13 ± 0.62*	1.26 (1.02, 1.55)

^a Subjects received 150 mg oral immediate-release racemic bupropion alone on the 3rd day of ritonavir (300 mg twice daily) treatment or on the 17th day of ritonavir (400 mg twice daily) treatment.

^b *C*_{max}, maximum concentration of drug in plasma; *T*_{max}, time to maximum concentration of drug in plasma; *t*_{1/2}, elimination half-life.

^c Parameter ratios (ritonavir/control) are the geometric means and the 90% confidence intervals about the geometric means (in parentheses). An asterisk indicates a value that is different from that for the control (*P* < 0.05).

ical implications, specifically with regard to the disposition of numerous CYP2B6 substrates, including antineoplastic agents, anesthetics and analgesics, drugs of abuse, antiretrovirals, pesticides, and other environmental contaminants, together encompassing as much as 8% of all therapeutic drugs (39, 49, 54). One example is the ritonavir induction of nelfinavir metabolism, evidenced by increased plasma ratios of metabolite M8/nelfinavir (1). Another notable example is methadone, a mainstay in the treatment of opiate addiction and acute and chronic pain. Ritonavir increases methadone clearance and can cause acute withdrawal (19, 30, 34, 38). Both short-term and steady-

state ritonavir treatments recently were shown to increase intravenous and oral methadone metabolism and clearance by 1.5- to 2-fold (30, 34). This occurred despite the profound ritonavir inhibition of CYP3A. The mechanism by which ritonavir induces methadone clearance is unknown. Methadone metabolism and clearance in vivo traditionally have been attributed to CYP3A (5, 10, 18) and ritonavir effects on methadone disposition to CYP3A induction (19, 38). Notwithstanding years of the clinical use of these drugs, the paradox of the ritonavir induction of methadone clearance despite CYP3A inhibition remains unexplained. Recent evidence suggests a

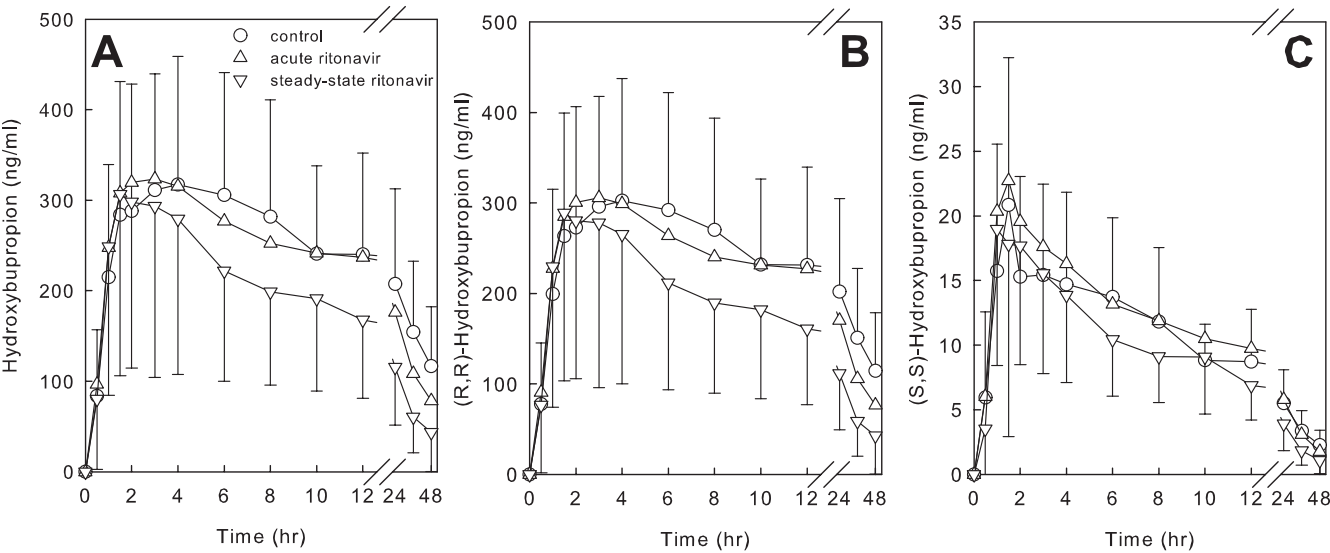


FIG. 2. Plasma concentrations of racemic hydroxybupropion (A) and hydroxybupropion diastereomers (B and C). Results are the means \pm SD ($n = 13$ for controls and acute ritonavir treatment; $n = 12$ for steady-state ritonavir treatment). Some SDs are omitted for clarity.

significant, if not predominant, role for CYP2B6 in methadone metabolism and clearance (20, 31, 47, 48). CYP3A inhibition failed to diminish methadone metabolism and clearance (30, 31, 34). Conversely, rifampin, which induces CYP2B6, increased methadone metabolism and clearance at a level similar to that by ritonavir. This suggests that the ritonavir induction of methadone metabolism and clearance may be mediated by hepatic CYP2B6 induction.

The clinical effects of ritonavir on human hepatic CYP2B6 are diametrically opposite to those on hepatic and intestinal CYP3A. Ritonavir is one of the most potent and efficacious mechanism-based inhibitors of CYP3A isoforms in vitro (13).

Clinically, acute and steady-state ritonavir treatments profoundly inhibit first-pass and hepatic CYP3A. Several recent investigations, using well-characterized CYP3A probes such as midazolam, triazolam, and alfentanil, demonstrated >95% inhibition of first-pass CYP3A activity by various ritonavir regimens, such as 100 to 200 mg/day, 200 mg twice daily, and 400 mg twice daily (6, 17, 22, 30, 34). Although early clinical investigations suggested that steady-state ritonavir treatment induced CYP3A based on the autoinduction of ritonavir clearance (29) and decreased ethinyl estradiol AUC and half-life (40), nevertheless it was recognized that such effects also could emanate from ritonavir induction of efflux transporters (6).

TABLE 2. Pharmacokinetic parameters for urine bupropion and hydroxybupropion

Parameter and substance	Results according to treatment type ^a				
	Control ($n = 13$)	Acute ritonavir ($n = 13$)		Steady-state ritonavir ($n = 12$)	
	Mean \pm SD	Mean \pm SD	Ratio	Mean \pm SD	Ratio
Apparent renal or formation clearance (ml/min) ^b					
Racemic bupropion	16 \pm 18	20 \pm 18	1.09 (0.76, 1.58)	21 \pm 28	1.11 (0.69, 1.79)
(R)-Bupropion	21 \pm 23	25 \pm 23	1.10 (0.75, 1.60)	28 \pm 42	1.09 (0.67, 1.78)
(S)-Bupropion	9 \pm 10	10 \pm 8	1.02 (0.71, 1.47)	10 \pm 10	1.14 (0.74, 1.76)
Hydroxybupropion	63 \pm 44	143 \pm 168*	1.78 (1.34, 2.36)	183 \pm 179*	2.09 (1.46, 2.98)
(R,R)-Hydroxybupropion	72 \pm 51	172 \pm 215*	1.78 (1.30, 2.42)	217 \pm 221*	2.07 (1.43, 2.99)
(S,S)-Hydroxybupropion	49 \pm 35	96 \pm 88*	1.75 (1.38, 2.21)	134 \pm 133*	2.12 (1.47, 3.06)
Hydroxybupropion/bupropion molar ratio at 0–24 h ^c					
Hydroxybupropion/bupropion	4.5 \pm 3.7	8.5 \pm 6.8*	1.76 (1.10, 2.81)	15.4 \pm 20.4*	2.04 (1.10, 3.77)
(R,R)-Hydroxybupropion/(R)-bupropion	4.1 \pm 3.4	7.7 \pm 6.2*	1.76 (1.08, 2.87)	14.7 \pm 20.0*	2.10 (1.10, 4.00)
(S,S)-Hydroxybupropion/(S)-bupropion	6.0 \pm 4.7	11.7 \pm 8.5*	1.83 (1.20, 2.80)	18.6 \pm 22.9*	1.95 (1.15, 3.31)

^a Subjects received 150 mg oral immediate-release racemic bupropion alone on the 3rd day of ritonavir (300 mg twice daily) treatment or on the 17th day of ritonavir (400 mg mg twice daily) treatment. Parameter ratios (ritonavir/control) are the geometric means and the 90% confidence intervals about the geometric means (in parentheses). An asterisk indicates that the value is significantly different from that for the control ($P < 0.05$).

^b Based on 0 to 48 h of urine collection.

^c Based on 0 to 24 h of urine collection.

The contradictory effects of ritonavir on human hepatic CYP2B6 and CYP3A activities are paradoxical, particularly since these isoforms share common regulatory pathways. The orphan nuclear receptors pregnane X receptor (PXR) and constitutive androstane receptor (CAR) both regulate the expression of CYP2B and CYP3A (51). Early investigations suggested that PXR and CAR were the predominant activators of CYP3A and CYP2B, respectively, although later findings of cross-talk between PXR and CAR demonstrated the reciprocal activation of their response elements (52). Cross-talk is asymmetrical, so that the activation of human PXR, which has a large and accommodating active site that binds numerous and diverse ligands (2, 11), results in the nonselective activation of both CYP2B6 and CYP3A4 (14). Conversely, human CAR, which has a smaller binding site (53), preferentially activates CYP2B6 over CYP3A4 (14). A recent screen of CYP2B6 and/or CYP3A4 inducers identified three classes: selective human PXR activators (i.e., rifampin), mixed human PXR and CAR activators (i.e., troglitazone), and preferential human CAR activators (i.e., efavirenz and nevirapine) (16). One possible explanation for reciprocal CYP2B6 induction/CYP3A inhibition by ritonavir is PXR activation combined with mechanism-based CYP3A inhibition. In human hepatocytes, ritonavir strongly activated PXR, upregulated CYP2B6 and CYP3A mRNA levels, and induced both CYP3A and CYP2B6 protein expression (8, 9, 15, 23, 37). Ritonavir was the most potent and efficacious PXR activator (15, 23), although it was not as efficacious in inducing CYP3A4 as predicted by PXR activation (23, 37). More importantly, while ritonavir induced both CYP2B6 and CYP3A4 protein expression, CYP3A activity was completely inhibited (8, 15, 37). Thus, the PXR-mediated coordinate upregulation of CYP2B6 and CYP3A4 expression by ritonavir was offset by the selective inhibition of CYP3A4 and not CYP2B6. Ritonavir is a potent ($K_i < 0.1 \mu\text{M}$) mechanism-based CYP3A4 inhibitor (13, 21). It appears to be a less potent inhibitor of CYP2B6 (50% inhibitory concentration, $2 \mu\text{M}$) (25). A similar in vivo scenario could result in the upregulation of both CYP3A4 and CYP2B6 expression, with the selective inhibition of CYP3A4 activity. Another possible explanation for the reciprocal clinical ritonavir induction of CYP2B6 and inhibition of CYP3A is that ritonavir is a preferential human CAR activator, resulting in selective CYP2B6 upregulation and likely also CYP3A4 inhibition. The antiretrovirals efavirenz and nevirapine have been characterized as preferential human CAR activators (16); however, the effects of ritonavir on CAR have not been reported.

One potential limitation of this investigation is that the steady-state ritonavir dose differs from that used in typical contemporary antiretroviral regimens (42). In early clinical use, ritonavir was dosed at 600 mg twice daily, although this dose was associated with considerable drug interactions and generally is no longer used for treating HIV-infected patients. Contemporary use is predominately in lower doses, typically 100 to 200 mg once or twice daily. The present protocol was designed as a mechanistic investigation specifically to provide insights into the mechanism of the ritonavir induction of methadone clearance in our previous investigation using the same dose of 400 mg twice daily (30, 34). Nonetheless, CYP2B6 induction occurred at higher (400 mg twice daily) as well as lower doses (after only 2 days of 200 to 300 mg twice daily) and

also after longer periods at 100 mg twice daily (27). Thus, the present investigation has both mechanistic and clinical relevance to ritonavir use at lower doses.

Ritonavir prescribing guidelines suggest that concomitant use with bupropion increases, or is predicted to increase, bupropion concentrations. Caution is advised if bupropion is administered with ritonavir, with a dosage reduction potentially being necessary (Norvir [ritonavir] soft gelatin capsules and oral solution prescribing information, Abbott Laboratories, North Chicago, IL). This may be based in part on extrapolation from the in vitro ritonavir inhibition of human liver microsomal bupropion hydroxylation (25). The present study demonstrates the need for caution in extrapolating in vitro observations and the necessity of performing clinical investigations to verify such observations, and it suggests that a revision of the ritonavir dosing guidelines is appropriate.

In summary, the present investigation shows that short-term (2 to 3 days) and steady-state ritonavir treatments increase the hydroxylation and clearance of the CYP2B6 probe (*S*)-bupropion, suggesting that ritonavir induces human CYP2B6 activity. CYP2B6 induction occurs despite the profound inhibition of CYP3A4 activity.

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REFERENCES

1. Aarnoutse, R. E., J. A. Droste, J. J. van Oosterhout, P. P. Koopmans, M. Popescu, P. Reiss, Y. A. Hekster, and D. M. Burger. 2003. Pharmacokinetics, food intake requirements and tolerability of once-daily combinations of nelfinavir and low-dose ritonavir in healthy volunteers. *Br. J. Clin. Pharmacol.* **55**:115–125.
2. Chang, T. K., and D. J. Waxman. 2006. Synthetic drugs and natural products as modulators of constitutive androstane receptor (CAR) and pregnane X receptor (PXR). *Drug Metab. Rev.* **38**:51–73.
3. Coles, R., and E. D. Kharasch. 2007. Stereoselective analysis of bupropion and hydroxybupropion in human plasma and urine by LC/MS/MS. *J. Chromatogr. B* **857**:67–75.
4. Coles, R., and E. D. Kharasch. Stereoselective metabolism of bupropion by CYP2B6 and human liver microsomes. *Pharm. Res.*, in press.
5. Crettol, S., J. J. Deglon, J. Besson, M. Croquette-Krokar, R. Hammig, I. Gothuey, M. Monnat, and C. B. Eap. 2006. ABCB1 and cytochrome P450 genotypes and phenotypes: influence on methadone plasma levels and response to treatment. *Clin. Pharmacol. Ther.* **80**:668–681.
6. Culm-Merdek, K. E., L. L. von Moltke, L. Gan, K. A. Horan, R. Reynolds, J. S. Harmatz, M. H. Court, and D. J. Greenblatt. 2006. Effect of extended exposure to grapefruit juice on cytochrome P450 3A activity in humans: comparison with ritonavir. *Clin. Pharmacol. Ther.* **79**:243–254.
7. Desta, Z., T. Saussele, B. Ward, J. Blievernicht, L. Li, K. Klein, D. A. Flockhart, and U. M. Zanger. 2007. Impact of CYP2B6 polymorphism on hepatic efavirenz metabolism in vitro. *Pharmacogenomics* **8**:547–558.
8. Dixit, V., N. Hariparsad, F. Li, P. Desai, K. E. Thummel, and J. D. Unadkat. 2007. Cytochrome P450 enzymes and transporters induced by anti-human immunodeficiency virus protease inhibitors in human hepatocytes: implications for predicting clinical drug interactions. *Drug Metab. Dispos.* **35**:1853–1859.
9. Dussault, I., M. Lin, K. Hollister, E. H. Wang, T. W. Synold, and B. M. Forman. 2001. Peptide mimetic HIV protease inhibitors are ligands for the orphan receptor SXR. *J. Biol. Chem.* **276**:33309–33312.
10. Eap, C. B., T. Buclin, and P. Baumann. 2002. Interindividual variability of the clinical pharmacokinetics of methadone: implications for the treatment of opioid dependence. *Clin. Pharmacokinet.* **41**:1153–1193.
11. Ekins, S., C. Chang, S. Mani, M. D. Krasowski, E. J. Reschly, M. Iyer, V. Kholodovych, N. Ai, W. J. Welsh, M. Sinz, P. W. Swaan, R. Patel, and K. Bachmann. 2007. Human pregnane X receptor antagonists and agonists define molecular requirements for different binding sites. *Mol. Pharmacol.* **72**:592–603.
12. Ekins, S., and S. A. Wrighton. 1999. The role of CYP2B6 in human xenobiotic metabolism. *Drug Metab. Rev.* **31**:719–754.
13. Ernest, Jr., C. S., S. D. Hall, and D. R. Jones. 2005. Mechanism-based inactivation of cytochrome P450 3A (CYP3A) by HIV protease inhibitors. *J. Pharmacol. Exp. Ther.* **312**:583–591.

14. Faucette, S. R., T. Sueyoshi, C. M. Smith, M. Negishi, E. L. Lecluyse, and H. Wang. 2006. Differential regulation of hepatic CYP2B6 and CYP3A4 genes by constitutive androstane receptor but not pregnane X receptor. *J. Pharmacol. Exp. Ther.* 317:1200–1209.
15. Faucette, S. R., H. Wang, G. A. Hamilton, S. L. Jolley, D. Gilbert, C. Lindley, B. Yan, M. Negishi, and E. L. LeCluyse. 2004. Regulation of CYP2B6 in primary human hepatocytes by prototypical inducers. *Drug Metab. Dispos.* 32:348–358.
16. Faucette, S. R., T. C. Zhang, R. Moore, T. Sueyoshi, C. J. Omiecinski, E. L. LeCluyse, M. Negishi, and H. Wang. 2007. Relative activation of human pregnane X receptor versus constitutive androstane receptor defines distinct classes of CYP2B6 and CYP3A4 inducers. *J. Pharmacol. Exp. Ther.* 320:72–80.
17. Fellay, J., C. Marzolini, L. Decosterd, K. P. Golay, P. Baumann, T. Buclin, A. Telenti, and C. B. Eap. 2005. Variations of CYP3A activity induced by antiretroviral treatment in HIV-1 infected patients. *Eur. J. Clin. Pharmacol.* 60:865–873.
18. Foster, D. J., A. A. Somogyi, K. R. Dyer, J. M. White, and F. Bochner. 2000. Steady-state pharmacokinetics of (R)- and (S)-methadone in methadone maintenance patients. *Br. J. Clin. Pharmacol.* 50:427–440.
19. Geletko, S. M., and A. D. Erickson. 2000. Decreased methadone effect after ritonavir initiation. *Pharmacotherapy* 20:93–94.
20. Gerber, J. G., R. J. Rhodes, and J. Gal. 2004. Stereoselective metabolism of methadone N-demethylation by cytochrome P4502B6 and 2C19. *Chirality* 16:36–44.
21. Granfors, M. T., J. S. Wang, L. I. Kajosaari, J. Laitila, P. J. Neuvonen, and J. T. Backman. 2006. Differential inhibition of cytochrome P450 3A4, 3A5 and 3A7 by five human immunodeficiency virus (HIV) protease inhibitors in vitro. *Basic Clin. Pharmacol. Toxicol.* 98:79–85.
22. Greenblatt, D. J., L. L. von Moltke, J. P. Daily, J. S. Harmatz, and R. I. Shader. 1999. Extensive impairment of triazolam and alprazolam clearance by short-term low-dose ritonavir: the clinical dilemma of concurrent inhibition and induction. *J. Clin. Psychopharmacol.* 19:293–296.
23. Hartley, D. P., X. Dai, J. Yabut, X. Chu, O. Cheng, T. Zhang, Y. D. He, C. Roberts, R. Ulrich, R. Evers, and D. C. Evans. 2006. Identification of potential pharmacological and toxicological targets differentiating structural analogs by a combination of transcriptional profiling and promoter analysis in LS-180 and Caco-2 adenocarcinoma cell lines. *Pharmacogenet. Genomics* 16:579–599.
24. Hesse, L. M., D. J. Greenblatt, L. L. von Moltke, and M. H. Court. 2006. Ritonavir has minimal impact on the pharmacokinetic disposition of a single dose of bupropion administered to human volunteers. *J. Clin. Pharmacol.* 46:567–576.
25. Hesse, L. M., L. L. von Moltke, R. I. Shader, and D. J. Greenblatt. 2001. Ritonavir, efavirenz, and nelfinavir inhibit CYP2B6 activity in vitro: potential drug interactions with bupropion. *Drug Metab. Dispos.* 29:100–102.
26. Hodgson, E., and R. L. Rose. 2007. The importance of cytochrome P450 2B6 in the human metabolism of environmental chemicals. *Pharmacol. Ther.* 113:420–428.
27. Hogeland, G. W., S. Swindells, J. C. McNabb, A. D. Kashuba, G. C. Yee, and C. M. Lindley. 2007. Lopinavir/ritonavir reduces bupropion plasma concentrations in healthy subjects. *Clin. Pharmacol. Ther.* 81:69–75.
28. Hsu, A., G. R. Granneman, G. Cao, L. Carothers, A. Japour, T. El-Shourbagy, S. Dennis, J. Berg, K. Erdman, J. M. Leonard, and E. Sun. 1998. Pharmacokinetic interaction between ritonavir and indinavir in healthy volunteers. *Antimicrob. Agents Chemother.* 42:2784–2791.
29. Hsu, A., G. R. Granneman, G. Witt, C. Locke, J. Denissen, A. Molla, J. Valdes, J. Smith, K. Erdman, N. Lyons, P. Niu, J. P. Decourt, J. B. Fourtillan, J. Girault, and J. M. Leonard. 1997. Multiple-dose pharmacokinetics of ritonavir in human immunodeficiency virus-infected subjects. *Antimicrob. Agents Chemother.* 41:898–905.
30. Kharasch, E. D., and C. Hoffer. 2004. Assessment of ritonavir effects on hepatic and first-pass CYP3A activity and methadone disposition using non-invasive pupillometry. *Clin. Pharmacol. Ther.* 75:P96.
31. Kharasch, E. D., C. Hoffer, D. Whittington, and P. Sheffels. 2004. Role of hepatic and intestinal cytochrome CYP3A and CYP2B6 in the metabolism, disposition and mitotic effects of methadone. *Clin. Pharmacol. Ther.* 76:250–269.
32. Kharasch, E. D., D. Mitchell, and R. Coles. 2008. Stereoselective bupropion hydroxylation as an in vivo phenotypic probe for cytochrome P4502B6 (CYP2B6) activity. *J. Clin. Pharmacol.* 48:464–474.
33. Kharasch, E. D., A. Walker, N. Isoherranen, C. Hoffer, P. Sheffels, K. Thummel, D. Whittington, and D. Ensign. 2007. Influence of CYP3A5 genotype on the pharmacokinetics and pharmacodynamics of the cytochrome P4503A probes alfentanil and midazolam. *Clin. Pharmacol. Ther.* 82:410–426.
34. Kharasch, E. D., A. Walker, D. Whittington, C. Hoffer, and P. Sheffels. 2008. Mechanism of ritonavir influence on methadone pharmacokinetics, pharmacodynamics, and clinical effect (abstract). *Clin. Pharmacol. Ther.* 83:540.
35. Kirchheiner, J., C. Klein, I. Meineke, J. Sasse, U. M. Zanger, T. E. Mürdter, I. Roots, and J. Brockmüller. 2003. Bupropion and 4-OH-bupropion pharmacokinetics in relation to genetic polymorphisms in CYP2B6. *Pharmacogenetics* 13:619–626.
36. Loboz, K. K., A. S. Gross, K. M. Williams, W. S. Liao, R. O. Day, J. K. Blievernicht, U. M. Zanger, and A. J. McLachlan. 2006. Cytochrome P450 2B6 activity as measured by bupropion hydroxylation: effect of induction by rifampin and ethnicity. *Clin. Pharmacol. Ther.* 80:75–84.
37. Luo, G., M. Cunningham, S. Kim, T. Burn, J. Lin, M. Sinz, G. Hamilton, C. Rizzo, S. Jolley, D. Gilbert, A. Downey, D. Mudra, R. Graham, K. Carroll, J. Xie, A. Madan, A. Parkinson, D. Christ, B. Selling, E. LeCluyse, and L. S. Gan. 2002. CYP3A4 induction by drugs: correlation between a pregnane X receptor reporter gene assay and CYP3A4 expression in human hepatocytes. *Drug Metab. Dispos.* 30:795–804.
38. McCance-Katz, E. F., P. M. Rainey, P. Smith, G. Morse, G. Friedland, M. Gourevitch, and P. Jatlow. 2004. Drug interactions between opioids and antiretroviral medications: interaction between methadone, LAAM, and nelfinavir. *Am. J. Addict.* 13:163–180.
39. Nolan, D., E. Phillips, and S. Mallal. 2006. Efavirenz and CYP2B6 polymorphism: implications for drug toxicity and resistance. *Clin. Infect. Dis.* 42:408–410.
40. Ouellet, D., A. Hsu, J. Qian, C. S. Locke, C. J. Eason, J. H. Cavanaugh, J. M. Leonard, and G. R. Granneman. 1998. Effect of ritonavir on the pharmacokinetics of ethinyl oestradiol in healthy female volunteers. *Br. J. Clin. Pharmacol.* 46:111–116.
41. Palovaara, S., O. Pelkonen, J. Uusitalo, S. Lundgren, and K. Laine. 2003. Inhibition of cytochrome P450 2B6 activity by hormone replacement therapy and oral contraceptive as measured by bupropion hydroxylation. *Clin. Pharmacol. Ther.* 74:326–333.
42. Piacenti, F. J. 2006. An update and review of antiretroviral therapy. *Pharmacotherapy* 26:1111–1133.
43. Piscitelli, S. C., D. R. Kress, R. J. Bertz, A. Pau, and R. Davey. 2000. The effect of ritonavir on the pharmacokinetics of meperidine and normeperidine. *Pharmacotherapy* 20:549–553.
44. Ramirez, J., F. Innocenti, E. G. Schuetz, D. A. Flockhart, M. V. Relling, R. Santucci, and M. J. Ratain. 2004. CYP2B6, CYP3A4, and CYP2C19 are responsible for the in vitro N-demethylation of meperidine in human liver microsomes. *Drug Metab. Dispos.* 32:930–936.
45. Robertson, S. M., S. R. Penzak, and A. Pau. 2007. Drug interactions in the management of HIV infection: an update. *Expert Opin. Pharmacother.* 8:2947–2963.
46. Rotger, M., S. Colombo, H. Furrer, G. Bleiber, T. Buclin, B. L. Lee, O. Keiser, J. Biollaz, L. Decosterd, and A. Telenti. 2005. Influence of CYP2B6 polymorphism on plasma and intracellular concentrations and toxicity of efavirenz and nevirapine in HIV-infected patients. *Pharmacogenet. Genomics* 15:1–5.
47. Totah, R. A., K. E. Allen, P. Sheffels, D. Whittington, and E. D. Kharasch. 2007. Enantiomeric metabolic interactions and stereoselective human methadone metabolism. *J. Pharmacol. Exp. Ther.* 321:389–399.
48. Totah, R. A., P. Sheffels, T. Roberts, D. Whittington, K. Thummel, and E. D. Kharasch. 2008. Role of CYP2B6 in stereoselective human methadone metabolism. *Anesthesiology* 108:363–374.
49. Turpeinen, M., H. Raunio, and O. Pelkonen. 2006. The functional role of CYP2B6 in human drug metabolism: substrates and inhibitors in vitro, in vivo and in silico. *Curr. Drug Metab.* 7:705–714.
50. Turpeinen, M., A. Tolonen, J. Uusitalo, J. Jalonen, O. Pelkonen, and K. Laine. 2005. Effect of clopidogrel and ticlopidine on cytochrome P450 2B6 activity as measured by bupropion hydroxylation. *Clin. Pharmacol. Ther.* 77:553–559.
51. Urquhart, B. L., R. G. Tirona, and R. B. Kim. 2007. Nuclear receptors and the regulation of drug-metabolizing enzymes and drug transporters: implications for interindividual variability in response to drugs. *J. Clin. Pharmacol.* 47:566–578.
52. Wei, P., J. Zhang, D. H. Dowhan, Y. Han, and D. D. Moore. 2002. Specific and overlapping functions of the nuclear hormone receptors CAR and PXR in xenobiotic response. *Pharmacogenomics J.* 2:117–126.
53. Windshügel, B., J. Jyrkkärinne, J. Vanamo, A. Poso, P. Honkakoski, and W. Sippl. 2007. Comparison of homology models and X-ray structures of the nuclear receptor CAR: assessing the structural basis of constitutive activity. *J. Mol. Graph Model.* 25:644–657.
54. Zanger, U. M., K. Klein, T. Saussele, J. Blievernicht, M. H. Hofmann, and M. Schwab. 2007. Polymorphic CYP2B6: molecular mechanisms and emerging clinical significance. *Pharmacogenomics* 8:743–759.